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Developing a rodent model for antipsychotic-induced metabolic adverse effects

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Contents

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2. Summary

Antipsychotic agents represent efficient therapy for serious psychiatric disorders, particularly schizophrenia, but also bipolar disorder, and are used by millions of patients worldwide. Metabolic adverse effects of antipsychotic drugs are thought to contribute significantly to the fact that life expectancy among schizophrenic patients is reduced with several decades. In particular, the so-called second-generation antipsychotics – most notably clozapine and olanzapine - significantly increase the prevalence of obesity, dyslipidemia, and type 2 diabetes. After initial cell culture experiments in our lab demonstrated that antipsychotic drugs activate lipid biosynthesis through the transcription factor SREBP, we set out to elaborate our findings in various preclinical model systems. Exposing glial-like and neuronal-like cultured cells to different antipsychotic agents, we showed that antipsychotics activated the expression of several SREBP-regulated genes encoding key enzymes in lipid synthesis with varying potency between the different drugs. The effects were much more potent in glial-derived than in neuron-derived cells, which is interesting in light of the fact that glial cells produce the bulk of lipids, essential in myelination and synaptic development, in the central nervous system.

We then treated female rats with the metabolically potent antipsychotic agent olanzapine or with aripiprazole, which is considered metabolically neutral in humans, for two weeks. Olanzapine induced marked increase in food intake and significant weight gain in rats. By including olanzapine-treated rats with restricted access to food, which did not gain weight, we demonstrated that weight gain primarily relies on increased food intake. Aripiprazole, included as a negative control, yielded significant increase in food intake and weight gain. Notably, increased serum triglyceride levels were detected in all olanzapine-treated rats, independent of weight gain, while serum triglyceride elevation was not present in rats treated with aripiprazole. In olanzapine-treated rats, serum triglyceride increase was accompanied by lipogenic activation in peripheral metabolic tissues, particularly in visceral adipose tissue.

In this 2-week experiment, we also included one treatment group receiving the modified fatty acid tetradecylthioacetic acid (TTA), a lipid-lowering agent, and one group treated with a combination of olanzapine and TTA. Despite olanzapine-induced weight gain in the olanzapine-TTA treatment group, TTA cotreatment led to significant reduction in lipid levels in serum and liver. In a follow-up experiment spanning 8 weeks, serum and lipid levels were similarly reduced in all rats receiving TTA, either as monotherapy or in combination with olanzapine or clozapine, in spite of weight-potentiating effects. In the liver, we found that TTA induced the transcription and activity of the key oxidative enzymes ACOX1 and CPT2, and downregulated transcription of HMGCR, the rate-limiting step in cholesterol synthesis. The effects of olanzapine monotherapy on food intake and weight gain wore off approximately three weeks into the experiment, and serum triglycerides were not elevated in olanzapine-treated after 8 weeks of treatment. Clozapine, unlike in humans, did not induce weight gain. We concluded that improved dosing regimens are necessary in order to maintain dysmetabolic effects of antipsychotic in rat in the long term and thus increase the relevance of this animal model. The concomitant weight gain potentiation and lipid-lowering effects of TTA, on the other hand, further supported the presence of independent mechanisms regulating body weight and lipid levels. These parameters may not be fully disconnected, however, as one potential mechanism suggested by us to underlie favourable lipid values was increased adipose tissue mass, providing storage capacity for surplus lipids.

3. List of publications

Paper I

Fernø J, Skrede S, Vik-Mo AO, Håvik B, Steen VM. Drug-induced activation of SREBP-controlled lipogenic gene expression in CNS-related cell lines: marked differences between various antipsychotic drugs. BMC Neurosci. 2006 Oct 20;7:69.

Paper II

Skrede S, Fernø J, Vázquez MJ, Fjær S, Pavlin T, Lunder N, Vidal-Puig A, Diéguez C, Berge RK, López M, Steen, VM. Olanzapine, but not aripiprazole, elevates serum triglycerides and activaties lipogenic gene expression in female rats. Int J Neuropsychopharmacol. 2011 Aug 19:1-17.

Paper III

Skrede S, Fernø J, Bjørndal B, Brede WR, Bohov P, Berge RK, Steen VM. Antipsychotic-induced metabolic adverse effects and pharmacological intervention: challenges with the female rat model (Manuscript).

4. Abbreviations

ACC	Acetyl-CoA carboxylase
AMPK	AMP-activated protein kinase
bHLH-Zip	basic-helix-loop-helix leucine zipper
CVD	cardiovascular disorder
DAG	diacylglycerol
DGAT	diacylglycerol acetyltransferase
ER	endoplasmatic reticulum
FASN	fatty acid synthase
GPAT	glycerol-3-phosphate acyltransferase
HMGCR	hydroxymethylglutaryl-Coenzyme A reductase
HMGCS1	hydroxymethylglutaryl-Coenzyme A synthase 1
Insig	Insulin-induced gene
MGAT	monoacylglycerol acyltransferase
PPAR	peroxisome proliferator-activated receptor
PGC1	peroxisome proliferator activated receptor gamma coactivator 1
SCAP	SREBP cleavage activating protein
SCD	stearoyl-CoA desaturase
SOAT	sterol O-acyltransferase
SREBP	sterol regulatory element binding protein
TZD	thiazolidinedione
TTA	tetradecylthioacetic acid

5. Introduction

5.1 Schizophrenia

5.1.1. Historical aspects

Schizophrenia is probably the psychiatric diagnosis surrounded by the most persistent mythical beliefs and the most resistant prejudice. In Norway, the adjective "schizophrenic" is quite frequently used in order to describe equivocal or inconsistent actions, statements or situations, demonstrating that members of the public confuse schizophrenia with the far less common dissociative identity disorder (formerly known as multiple personality disorder). The fact that the term "schizophrenia" originally means "split mind" may have contributed to this misconception. When Eugene Bleuler introduced the term in 1908, he used the German words "Zerreißung" (tearing) and "Spaltung" (splitting) in order to describe the core concept of a group of "syndromes" (i.e., constellations of symptoms) characterized, among other pathological manifestations, by disintegrated psychological association processes [1]. He elaborated on the concept of "splitting" in his famed 1911 work on schizophrenic disorders, "Dementia praecox oder gruppe der schizophrenien", and defined several other classic symptoms of schizophrenia, such as psychotic symptoms ("a predilection for fantasy"), disrupted affective abilities and "autism", a severe loss of interest in the surroundings [1].

5.1.2 Clinical manifestations

At present, the most commonly used diagnostic criteria for schizophrenia are found in the diagnostic manuals published by the American Psychiatric Association (Diagnostic and Statistical Manual of Mental Disorders, 4th edition - DSM-IV), or by

the World Health Organization (International Statistical Classification of Diseases and Related Health Problems, 10th revision - ICD-10) [2, 3]. DSM-IV lists 5 diagnostic subgroups for schizophrenia, with the common features of "disturbances in thought, perception, affect, behaviour, and communication that last longer than 6 months." In addition, patients must exhibit so-called "active phase symptoms" for at least 1 of these 6 months (unless successfully treated). Active phase symptoms include psychotic symptoms, such as hallucinations (often auditory) and delusions, odd beliefs, or bizarre perceptual experiences. Such symptoms, representing features not seen in healthy individuals, are often characterized as "positive symptoms". In the other end of the spectrum, "negative symptoms" are also hallmarks of schizophrenia. This term is used to describe the absence of emotions, thoughts or behaviour desirably present in healthy individuals, and may manifest as social withdrawal, affective flattening, apathy, or anhedonia. Patients may present with additional symptoms such as disorganized speech and/or disorganized or catatonic behaviour. Impairment of cognitive capabilities is also recognized as an important aspect of schizophrenia. Most patients with schizophrenia experience recurring psychotic episodes throughout their lives.

5.2 Epidemiological aspects of schizophrenia

5.2.1 Incidence and prevalence

The lifetime risk of schizophrenia has traditionally been given at ~1% worldwide. Estimates of incidence (i.e., the number of new cases in a given population per year) depend on a large number of factors such as diagnostic criteria, the diagnostic methods used, the organisation of local health care systems, and demographic elements such as general mortality and migration [4]. Thus, incidence estimates vary between studies. Stringent diagnostic criteria yielded incidence rates ranging from

6/100,000 to 14/100,000 in a large multinational WHO study (the so-called 10-country study) [5]. One of the conclusions in this study was that the incidence of schizophrenia shows little variation across populations. However, the question of whether the 10-country study was designed in a way that would ensure the detection of differing incidence between populations has been raised, and the results sparked much debate [6]. Recently, a meta-analysis indicated an overall median incidence of schizophrenia of 15,2 per 100,000, with an estimated 7 out of 1000 individuals diagnosed with the disorder at some point in their life [7]. This and other studies indicate that average lifetime prevalence across all populations may be slightly lower than the conventional 1% estimate, and that schizophrenia may be less uniformly distributed than previously thought [7-9]. Furthermore, contrasting former beliefs of even gender distribution, meta-analyses have revealed that the male:female risk ratio of developing schizophrenia may be ~ 1.4:1 [10, 11].

5.2.2 Costs of schizophrenia

Due to factors such as early symptom debut (i.e., early twenties), protracted course and complex treatment schemes, the economic burden of schizophrenia is overwhelming. An estimated 1,5-3% of health care and social spending of developed countries is accounted for partly by direct costs, such as expenses for treatment, and partly by indirect costs (e.g., lost productivity) of schizophrenia [12]. In addition, non-quantifiable losses of social and psychological character affect patients and family members alike. In statistics generated by the WHO, schizophrenia is listed as the 5th and 6th most significant cause of years lived with disability (YLD) in men and women, respectively [13].

5.2.3 Risk factors for schizophrenia

Twin and adoption studies have provided clear evidence that the heritability of schizophrenia is high, perhaps as high as 80% according to one meta-analysis [14]. Conventionally, a heritable, "intrinsic" vulnerability is thought to coincide with "external" risk factors to trigger the onset of the disorder in an individual. Generally accepted "external" risk factors include being born during the winter, high paternal age, obstetric complications, prenatal viral infections, and cannabis use [8]. The disorder is often characterized as "multifactorial", meaning that several circumstances must coexist in order to trigger symptoms.

Conscious of the high heritability, researchers have attempted to identify susceptibility genes for schizophrenia, i.e. genes in which defects (mutations) could increase the risk of suffering from the disorder. The Schizophrenia Research Forum (http://www.schizophreniaforum.org) maintains a list of the genes presently found to have the strongest association with schizophrenia. The latest ranking (April 2011) is topped by the genes PRSS16 (PRSS16 protease, serine, 16), PGBD1 (piggyBac transposable element derived 1), and NRGN (neurogranin (protein kinase C substrate, RC3) [15].

5.2.4 Neurochemical paradigms of schizophrenia

The pathophysiology of schizophrenia, despite eager research, remains elusive. The most influential paradigm in neuromolecular schizophrenia research during the last 40 years has undoubtedly been the so-called dopamine hypothesis. Early versions of this theory were based on the fact that several drugs relieving schizophrenic symptoms bind to, and block, dopamine receptors (particularly D_2 receptors) in the brain, as discussed below [16]. Thus it was suggested that cerebral dopaminergic "overdrive" is an essential component of the pathophysiology of schizophrenia. Later versions of the dopamine hypothesis proposed differential dopaminergic dysfunction in neuronal

subpopulations, with elevated mesolimbic (subcortical) dopaminergic signalling possibly underlying positive symptoms, and reduced dopaminergic activity in the prefrontal cortex theoretically causing negative symptoms [17]. It has been suggested that the prefrontal hypodopaminergic state may actually cause an increase in striatal dopaminergic signalling [17]. More recently, dysfunction in other neurotransmitter systems, such as the glutamatergic and GABAergic systems, have also been implicated [16]. Possible defects in several aspects of neuronal signalling are integrated in the so-called neurodevelopmental hypothesis, which focuses on embryonic/developmental defects in synaptic density and other aspects of neuronal function [16]. Demyelination, i.e. loss of myelin, the primary component of white matter, affects neuronal connectivity and is thought to be of significance in the pathophysiology of schizophrenia [18, 19]. In the CNS, myelin is synthesized by oligodendrocytes, a type of glial cells embedding neurons, to facilitate neuronal conductivity [20]. Signs of impaired myelination have been demonstrated in patients with schizophrenia [18, 21, 22], and may either result from reduced myelination during late adulthood or degenerative processes during the course of the illness itself. Most relevant studies include schizophrenic patients receiving pharmacological treatment for schizophrenia, an important confounder which is difficult to avoid, but often deemphasized in the interpretation of results.

5.3 Antipsychotic drugs

5.3.1. Early history of pharmacological therapy for psychiatric disorders

Historically, medicine had little to offer patients suffering from psychosis or other severe psychiatric symptoms. Often, treatment was characterized by more or less desperate attempts to ameliorate suffering and prevent patients from inflicting injury on themselves or others. Methods of treatment such as lobotomy, insulin shocks (the

induction of a hypoglycaemic state leading to loss of consciousness) or "sleep cures" (prolonged comatose states induced by barbiturates or similar agents) were employed during the first half of the 20th century [23, 24]. Treatment attempts such as these, which may seem primitive and ill-considered to us, must be viewed in light of the scarce options available at the time. Some patients actually appear to have improved, or at least to have experienced blunted positive symptoms, after receiving unspecific pharmacological treatment employed in order to achieve general sedation [24, 25]. Nevertheless, the fact remains that many types of treatment administered to the mentally ill caused considerable harm or even fatal outcome; for instance, "sleep cures" had a 5% mortality rate [24].

Throughout the 1950s, several pharmacological agents specifically improving psychotic symptoms were introduced, and rapidly made their way into clinical practice. Reserpine, an alkaloid isolated from the dried root of the shrub *Rauwolfia serpentina*, predated the drugs presently regarded as antipsychotic agents. In India, *Rauwolfia serpentina* was reportedly used to treat "insanity" long before being introduced to the Western world as an antihypertensive agent in the late 1940s [26]. Indeed, in addition to its antihypertensive properties, reserpine was found to possess antipsychotic properties [27]. However, reserpine never gained widespread use as an antipsychotic agent due to unacceptable side effects (hypotension and, importantly, depression [28]), and due to the introduction of other pharmacological agents with antipsychotic properties.

5.3.2 First-generation antipsychotics

The first specific antipsychotic agent was chlorpromazine, a phenothiazine synthesized in 1950 [26]. Commercially introduced as a treatment for psychiatric illnesses in 1953, chlorpromazine is considered the prototype antipsychotic agent, and the first of the so-called "typical", or first-generation, antipsychotics [29]. Its introduction has been described as a revolution by psychiatrists who, for the first time, observed specific treatment-induced regression of positive symptoms in patients with

schizophrenia [25]. Haloperidol, synthesized in 1958 and commercially launched in Europe in 1959, belongs to a different chemical class than chlorpromazine, namely the butyrophenones [29]. Several other antipsychotics were also introduced during the 1950s and 1960s (Table 5.1). During the 1970s, experiments revealed that all antipsychotic agents known thus far were characterized by high affinity for dopaminergic receptors, blocking such receptors in the brain and thereby inhibiting the binding of dopamine [30]. In particular, dopamine D_2 receptor antagonism seemed essential in terms of antipsychotic effect; drugs lacking this property have later been demonstrated to have inferior effect on psychotic symptoms [31, 32]. Chlorpromazine is a relatively weak D_2 antagonist compared to other early antipsychotics, while haloperidol is a potent D_2 blocker [33, 34].

Drug	**Chemical group**	**Commercially introduced**	**Current trade names** [1]
Chlorpromazine	Phenothiazine	1953	(Largactil)[2]
Perphenazine	Phenothiazine	1957	Trilafon
Haloperidol	Butyrophenone	1959	Haldol
Zuclopenthixol	Thioxanthene	1962	Cisordinol

Table 5.1 Selected first-generation (typical) antipsychotics [35].

The correlation of D_2 affinity with antipsychotic effect is now well established; D_2 occupancy above a certain threshold is required in order to achieve clinical antipsychotic effect [36]. However, several dopaminergic pathways with physiologically

[1] Norway

[2] Not for standard sale in Norway (2011)

distinct functions exist in the brain, and D_2 occupancy yields site-specific clinical effects [32]. During clinical trials and early clinical use, it became evident that both chlorpromazine, haloperidol and other typical antipsychotic agents induce severe dose-dependent extrapyramidal side effects, including akathisia (an intense feeling of restlessness or unease), Parkinsonism, dystonias, and tardive dyskinesia [34, 37]. The propensity to induce these adverse effects is correlated with D_2 affinity in the striatum, and dopaminergic blockade in this area of the brain is thought to be the main cause of extrapyramidal side effects [32].

5.3.3 Second-generation antipsychotic agents

The serious adverse effects associated with typical antipsychotic agents encouraged the search for new antipsychotics. Clozapine, the first of the so-called second-generation antipsychotic agents, was synthesized in 1958 (i.e., the same year as haloperidol) patented in Schwitzerland in 1960, but not introduced clinically in Europe until 1972, and in the USA in 1990 (reviewed in [38, 39]). Second-generation antipsychotics are frequently designated “atypical” and viewed as a group, despite pharmacological heterogeneity. In general, the most distinct differences between first- and second-generation drugs result from variations in D_2 and serotonin (5-hydroxytryptamine, 5-HT) receptor affinity. Several second-generation agents occupy 90-100% of 5-HT_2 receptors, with 5-HT_{2A} antagonism not observed for typical agents, while the degree of D_2 blockade is generally lower than among the typical antipsychotics [32, 36]. Accordingly, the risk of extrapyramidal side effects is significantly lower in patients treated with second-generation than in those treated with first-generation antipsychotics. For instance, clozapine binds D_2 receptors much more weakly than do first-generation drugs, while its affinity for serotonergic receptors (5-HT_{2A}, 5-HT_{2C}) is as much as 20 times higher than its D_2 affinity [31, 36, 40]. Unfortunately, treatment with clozapine carries a risk of agranulocytosis (a sharp decline in the number of circulating white blood cells, with resultant risk of infection), a potentially lethal adverse effect [41]. After being withdrawn from the

market in 1975 due to the risk of agranulocytosis, clozapine was relaunched in the USA in 1990 after clinical studies demonstrated its superiority over typical antipsychotic agents in treatment-resistant cases of schizophrenia [42]. At present, however, other atypical drugs are commonly regarded as primary choices in newly diagnosed psychoses.

Drug	**Chemical group**	**Commercially introduced**	**Current trade names (Norway)**
Clozapine	Dibenzodiazepine	1972	Leponex, Clozapin, Clozapine
Risperidone	Benzisoxazole	1994	Risperdal, Risperidon
Olanzapine	Thienobenzo-diazepine	1996	Zyprexa, ZypAdhera, Olanzapin
Ziprasidone	Benzisothiazolyl	2001	Zeldox
Aripiprazole	Quinolone	2002	Abilify
Quetiapine	Dibenzothiazepine	1998	Seroquel, Quetiapin
Amisulpride	Benzamide	1990	Solian

Table 5.2 Second-generation (atypical) antipsychotics [35]. Aripiprazole has been called the first "third-generation" antipsychotic due to its properties as a partial D_2 agonist.

Olanzapine, approved by the American Food and Drug Administration (FDA) in 1996, is chemically related to clozapine [43]. Reminiscent of clozapine's properties, olanzapine's affinity for 5-HT_2 receptors exceeds its affinity for D_2 receptors, with an *in vitro* 5-HT_2/D_2 affinity ratio approximating 12 [40]. Imaging studies have

demonstrated that olanzapine's D_2 affinity is higher than that of clozapine [36]. Agranulocytosis has rarely been reported in patients treated with olanzapine [44]. However, relatively soon after the drug's introduction, reports of severe metabolic side effects surfaced; these effects are discussed below [45]. Ziprasidone, marketed since 2001, is another atypical antipsychotic, with D_2 affinity comparable to that of risperidone as well as high affinity for several 5-HT receptors, combined with serotonin and noradrenaline reuptake inhibition [46]. Aripiprazole, commercially available from 2002, is frequently referred to as the first "third-generation" antipsychotic. This agent's pharmacological properties deviate from that of prior antipsychotics in that it is a partial D_2 agonist (or possibly possessing "functionally selective" D_2 affinity) rather that a "traditional" D_2 antagonist [47]. Aripiprazole also possesses partial agonism at 5-HT_{1A} receptors, as well as 5-HT_2 antagonism.

	5-HT1a	5-HT2A	5-HT2C	D1	D2	α2C	αA1	α1B	β2	M1	M3	H1
Chlorpromazine	(+)	+++	++	+	+++	++	++++	++++	+	++	++	++++
Haloperidol	(+)	++	0	++	+++	+	++	+++	0	0	0	(+)
Risperidone	+	++++	++	+	+++	+++	+++	+++	0	0	0	++
Olanzapine	(+)	+++	++	++	++	++	+	+	0	++	++	+++
Clozapine	+	++	++	+	+	++	+++	+++	0	++	++	+++
Quetiapine	+	+	(+)	+	+	++	++	++	(+)	+	(+)	+++
Aripipazole	+++	+++	+	+	++++	++	++	++	0	(+)	(+)	++
Ziprasidone	++	+++	++	++	+++	++	++	+++	(+)	0	0	+

Table 5.3 Receptor binding profiles of various antipsychotic agents. 0: no affinity; (+) very weak affinity; + weak affinity; ++ intermediate affinity; +++ strong affinity; ++++ very strong affinity for the receptor subtype, reflected in darkening colour gradient. Adapted from [31]. 5-HT: 5-hydroxytryptaminergic (=serotonergic); D: dopaminergic; α,β: subtypes of adrenergic receptors; M: muscarinic, H: histaminergic.

5.3.4 Metabolic adverse effects of antipsychotic drugs

Metabolic disturbances, including weight gain, are recognized adverse effects of typical antipsychotic drugs, both phenotiazines and, to a moderate degree, haloperidol, as reviewed in [48, 49]. Glucose dysregulation, occasionally debuting as ketoacidosis, was also observed in patients treated with typical antipsychotics [50, 51], and increased serum cholesterol was described in patients treated with chlorpromazine in 1967 [52]. With increasing use of clozapine and olanzapine, however, it soon became evident that these antipsychotics induce more frequent and more serious metabolic dysfunction than older drugs, and this issue has gained increasing attention during the last decades. Early clinical studies on clozapine mention weight gain as an adverse event [53, 54]. Several years later, elevated serum triglyceride levels were reported in patients treated with clozapine [55-57]. Reports on olanzapine's adverse effect profile published in the late 1990s, while describing low risk of dyskinesias and hematotoxicity, also mention the risk of weight gain [58, 59], later demonstrated to occur due to increased adipose tissue mass [60, 61]. For olanzapine and clozapine, a frequently cited meta-analysis estimated an average short-term weight gain (10 weeks) in the range of 3.5-4 kg, with continued weight gain at least during the first year of treatment – in one study, 80% of first-episode psychotic patients receiving olanzapine gained >7% of pre-treatment body weight during the first 52 weeks of treatment [62].

During the early years of olanzapine availability, the implications of metabolic adverse effects remained unclear ("the significance of this [i.e., weight gain] beyond cosmetic effects is unknown" [63]), but the first reports of olanzapine-induced hypertriglyceridemia were published during the same period [63-65]. Average olanzapine-induced increase in serum triglycerides is often given at 30-50%, while increase in serum cholesterol levels has also been reported during clozapine and olanzapine treatment [57, 65, 66]. Furthermore, both clozapine and olanzapine have been demonstrated to increase the risk of insulin resistance and type 2 diabetes [66-69]. Consequently, atypical antipsychotics significantly increase the risk of developing the constellation of parameters often termed the metabolic syndrome (Table 5.4).

Parameter	Males	Females
Waist circumference	≥94 cm*	≥88 cm*
Serum triglycerides	>1,7 mmol/l	>1,7 mmol/l
Serum HDL	<1,03 mmol/l	<1,29 mmol/l
Blood pressure	Systolic >130 or diastolic >85 mmHg	
Fasting serum glucose	> 5,6 mmol/l or recognized type 2 diabetes	

Table 5.4. Patients with central obesity plus any two of the findings described above fulfil the criteria for the diagnosis of metabolic syndrome [70]. * Europids.

The risk of weight gain, serum lipid increase and glucose dysregulation is generally regarded as intermediate for the second-generation agents risperidone and quetiapine, and low for aripiprazole and ziprasidone [49, 62, 65, 69, 71-73]. In fact, replacing olanzapine with aripiprazole has been shown to significantly improve the metabolic status of patients [74].

5.3.5 Clinical implications of metabolic adverse effects

Mortality rates among patients with schizophrenia are markedly increased compared to those found in the general population, causing patients with serious mental disorders to lose 2-3 decades of life on average [7]. This is partly due to increased suicide rates and increased susceptibility to fatal accidents, but most importantly due to early death from somatic conditions, with cardiovascular disorders as the single most common cause of death [7, 75, 76]. Compared to the general population, patients with psychiatric disorders may have a higher background risk of developing the metabolic syndrome, which may lead to cardiovascular disorders [77, 78]. Failure to seek

medical care or attend screening programmes, life style issues among patients (smoking, sedentary life style), and inadequate attention from caregivers concerning somatic comorbidity are probably important causes [75, 76, 79]. This complicates the interpretation of data regarding the contribution of antipsychotics to metabolic risk, particularly as some reports on metabolic dysfunction in schizophrenic patients include patients having received antipsychotic agents [80]. However, numerous studies indicate that treatment with antipsychotics adds significantly to the mortality rates in patients with serious mental disorders [69, 75, 81, 82]. Metabolic dysfunction, particularly weight gain, is also a potential cause of non-adherence, increasing the risk of psychotic relapse [83].

In addition to patients with schizophrenia, many individuals diagnosed with other psychiatric disorders, e.g. bipolar disorder, may respond well to antipsychotic drugs [84]. According to Eli Lilly's 2008 sales figures, the company made $4.7 billion from worldwide sales of olanzapine that year [85]. In Norway, official sales figures show that 15,649,516 DDD (estimated average daily dose for an adult patient) of antipsychotic agents were prescribed in Norway in 2008 [86]. Consequently, a very large number of patients worldwide receive antipsychotic treatment and are thus at risk of developing metabolic adverse effects.

5.3.6 Can receptor binding profiles explain metabolic adverse effects?

Increased food intake, primarily due to impairment of satiety onset, is thought to be the main underlying cause of weight gain induced by antipsychotic agents, and has been demonstrated both in humans [81, 87] and in rodents [88-90]. At present, no consensus exists in terms of the pharmacological properties underlying hyperphagia and other metabolic adverse effects, or the intracellular signalling pathways through which they are mediated. Several antipsychotic agents have antihistaminergic properties [31], and affinity for histaminergic (H_1) receptors correlates with weight gain [91, 92]. H_1 antagonism is linked to increased food intake [91], suggestedly through H_1-mediated activation of AMP-activated protein kinase (AMPK) in the hypothalamus [93]. The

involvement of several other receptors (serotonergic 5-HT_{2C}, adrenergic α1 and α2 receptors, and muscarininc M3 receptors) has also been implicated in antipsychotic-induced weight gain [89]. The complex receptor binding profiles of antipsychotic drugs (Table 5.3) complicate the identification of one or several receptors primarily responsible for weight gain [94], and predictions of weight gain risk based on receptor binding profiles are sometimes unsuccessful. For instance, ziprasidone, which is recognized not to induce significant weight gain in humans, possesses both 5-HT_{2C} antagonism (high) and H_1 affinity (moderate) [95], and would thus be expected to induce weight gain. Notably, some antipsychotics with weak affinity for H_1 receptors, e.g. haloperidol, are known to cause moderate weight gain [31, 49, 91]. Thus, a well-defined receptor binding profile resulting in increased risk of weight gain has yet to emerge. Regarding other dysmetabolic adverse effects, H_1, 5-HT_{2C}, and M_3 receptors has been linked to derangements in glucose metabolism, while no receptor binding profile has been defined as far as dyslipidemia is concerned [96, 97].

5.3.7 Animal models for antipsychotic-induced metabolic adverse effects

In the exploration of the molecular mechanisms underlying metabolic adverse effects, a reliable animal model is instrumental. Rodent models of antipsychotic treatment have been extensively explored, with two major challenges surfacing during the two last decades. Firstly, the degree to which each specific antipsychotic agent induces metabolic side effects differ, in some cases, between human and rodent, particularly with regard to weight gain. Secondly, in rodents, antipsychotic-induced weight gain is sex-dependent, i.e. observed almost exclusively in females (Table 5.5). Conclusive evidence for gender differences in the risk of developing antipsychotic adverse effects has not been found in humans [98, 99]. As described above, olanzapine and clozapine are the antipsychotic agents most prone to induce massive weight gain and related dysmetabolic features, such as dyslipidemia, in patients [49, 51, 100, 101]. In female rats, elevated food intake and weight gain through increased adipose tissue mass during

short-term treatment with olanzapine (1-10 mg/kg) are well characterized, even in animals receiving standard rodent chow with high carbohydrate and low fat content [89, 102, 103]. Olanzapine has also been demonstrated to have hyperphagic effects in male rats [88, 104, 105]. Furthermore, studies in male rats have shown that subchronic treatment with olanzapine increases adipose tissue mass, but not body weight, using diets with medium to high fat content [106, 107]. One study in which olanzapine-treated male rats received standard laboratory chow also reported increased adipose tissue mass in the absence of hyperphagia and body weight gain after 20 days of olanzapine treatment [105].

As for clozapine, with a clinical metabolic profile similar to that of olanzapine [69], hyperphagia has been reported in male rats receiving a clozapine dose of 0.3 mg/kg [88]. Weight gain has not been demonstrated in rats of either gender treated with 0.5-8 mg/kg [108, 109], but was reported in one 28-day study in female rats treated with 20 mg/kg clozapine [110]. In contrast, clozapine has somewhat unexpectedly been reported to induce weight reduction in rats at doses of 6-10 mg/kg [109, 111]. Reminiscent of observations from olanzapine-treated male rats, clozapine treatment has been shown to induce adiposity in female rats, with no effect on weight gain, except in one study reporting weight gain in male rats receiving clozapine 20 mg/kg for 7 weeks [111, 112]. Aripiprazole, considered metabolically neutral in patients, has been demonstrated in one study to induce moderate weight gain in female rats (8 mg/kg) [103], while apparently weight-neutral in a similar experiment using aripiprazole a dose of 2.25 mg/kg [113]. In agreement with the latter report, an aripiprazole dose of 2 mg/kg failed to induce hyperphagia in female rats [114]. Ziprasidone, also regarded metabolically neutral in patients, has not been demonstrated to possess hyperphagic effect in rat, although some groups have reported moderate weight gain in female rats at relatively low doses (2-10 mg/kg) [115-117].

	Olanzapine		**Clozapine**		**Aripiprazole**		**Ziprasidone**	
	♂	♀	♂	♀	♂	♀	♂	♀
Weight gain	↔ [105-107, 118] ↑ [119]	↑ [89, 102, 103, 118]	↔/↓ [108, 109, 118] ↑ [112]	↔/↓ [108, 109, 111, 118] ↑ [110]		↑ [103] ↔ [113]	↔ [106]	↑ [117]
Adipose mass	↑ [105-107]	↑ [103]		↑ [111]		↔ [113]	↑ [106]	
Hyperphagia	↑ [88, 105] ↔ [105]	↑ [103, 120]	↑ [88] ↔ [118]	↔ [111, 118]		↔ [114]	↔ [106]	
Serum triglycerides		↔ [103, 115, 121]					↔ [106]	↔ [115]
Glucose dysmetabolism	↑ [122, 123]	↑ [124]	↑ [122, 123]				↔ [122, 123]	

Table 5.5 Overview of dysmetabolic features demonstrated in rats. ↑: increase observed relative to vehicle. ↔: no change observed relative to vehicle. ↓ decrease observed relative to vehicle.

Few studies have reported lipid levels in rodent experiments. Serum triglycerides have largely been reported as unaltered by olanzapine [103, 115, 121], while increased serum free fatty acids after treatment with this drug have been shown in one experiment [103]. Derangements in glucose metabolism have been thoroughly demonstrated in rats treated with olanzapine and clozapine [122-124]. In female mice, the same pattern as in rats, with olanzapine-induced weight gain, has been demonstrated [125]. A few studies have also shown olanzapine-induced increase in serum triglycerides in female mice [126, 127].

5.4 Lipid metabolism

5.4.1 General aspects of lipid metabolism

Lipids constitute a large group of molecules involved in numerous essential processes and structures in the human organism. Fatty acyls (fatty acids), mono-, di-, and triglycerides, phospholipids and sterol-containing molecules, such as cholesterol, all belong to this class of macromolecules. A short overview of relevant aspects of lipid metabolism is presented below.

5.4.2 Free fatty acids and triglycerides

In times of excess, energy is primarily stored as triglycerides in adipose depots [128]. Lipids may be absorbed from the diet or synthesized *de novo* from pyruvate [128]. *De novo* synthesis primarily occurs in the liver, from which triglycerides are exported to white adipose tissue. The committed step in fatty acid synthesis is the formation of malonyl-CoA from acetyl-CoA, synthesized by acetyl-CoA carboxylase 1 (ACC1) (Figure 5.1) [128]. Malonyl-CoA then undergoes elongation in several steps catalyzed by fatty acid synthase (FASN), which possesses 7 enzymatic sites [128]. FASN synthesises palmitate (16:0), a 16-carbon, saturated fatty acid (i.e., lacking double bonds). Palmitate may be further elongated by elongases, and/or desaturated by desaturases, enzymes introducing double bonds. The desaturase most relevant to this thesis is stearoyl-CoA desaturase (SCD1), a Δ9 desaturase introducing double bond between C9 and C10 to yield, if palmitate is the substrate, palmitoleate [C16:1(Δ^9)] [128].

Three fatty acyl-CoA molecules linked to a glycerol-derived backbone (glycerol-3-phosphate) form a triglyceride molecule. Two of the three carbon sites of the glycerol backbone is acetylated in a reaction catalyzed by glycerol-3-phosphate acyltransferase (GPAT; Figure 5.1) and monoacylglycerol acyltransferase (MGAT), forming

phosphatidic acid [129]. GPAT constitutes the committed step in triglyceride synthesis. After dephosphorylation of phosphatidic acid, yielding diacylglycerol (DAG), DAG is acetylated by diacylglycerol acetyltransferase (DGAT), producing triacylglyceride (triglyceride) [129].

The anabolic hormone insulin is necessary for both triglyceride synthesis and for energy uptake and storage in adipose depots; lipid uptake to adipose tissues depends on maintained insulin sensitivity [128]. Abnormalities in lipid metabolism and glucose dysregulation are intimately related, and obesity is closely correlated with insulin resistance [130].

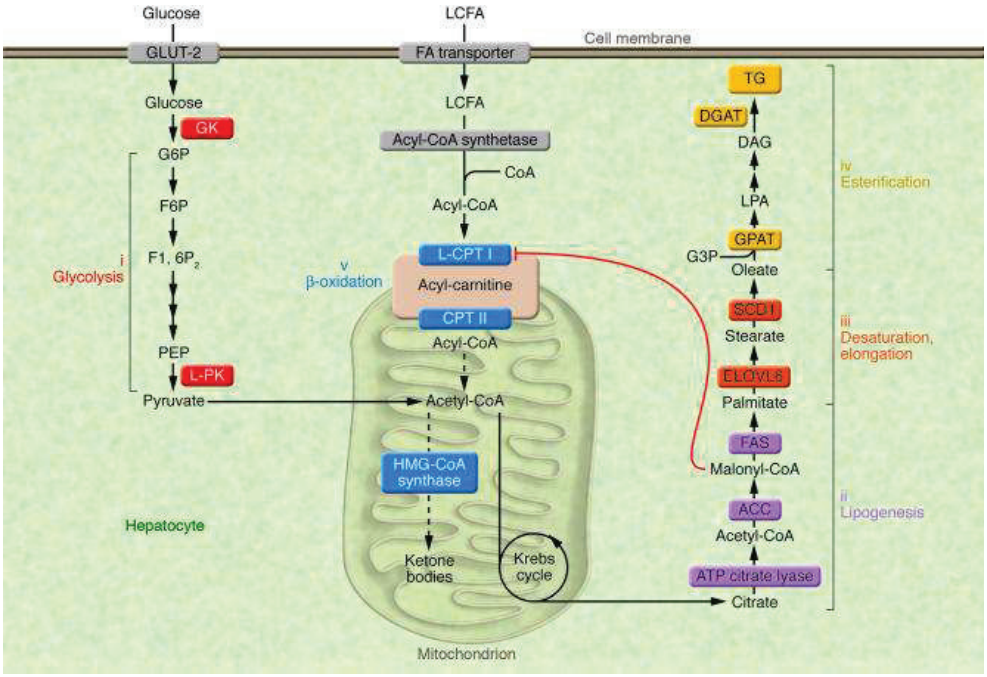

Figure 5.1 Important steps in fatty acid synthesis (purple), desaturation (orange), triglyceride biosynthesis (yellow) and fatty acid oxidation (blue). Taken from [131], with permission. For full names of relevant enzymes, see text.

5.4.3 Cholesterol metabolism

The percentage of cholesterol in cellular membranes has significant influence on the physical properties and organization of the membrane [132, 133]. In addition, cholesterol is a substrate for synthesis of complex sterols, such as steroid hormones (e.g. cortisol, testosterone and estradiol), and bile acids [134]. Like fatty acids and triglycerides, cholesterol may be absorbed from the diet or synthesized *de novo* in the liver. *De novo* synthesis of cholesterol is a complex pathway, with hydroxymethylglutaryl-Coenzyme A reductase (HMGCR) as the rate-limiting enzyme [128]. Statins, commonly used lipid-lowering drugs, are inhibitors of HMGCR [128]. For transport and storage, cholesterol is esterified, i.e., linked to fatty acids through an ester binding, possible because cholesterol contains an –OH group. Esterification, which decreases cholesterol's lipophilic properties, is catalyzed by the enzyme sterol O-acyltransferase (SOAT, also known as ACAT).

5.4.4 Lipids in the brain

As mentioned in section 5.2.4, glial cells (oligodendrocytes) produce myelin embedding the axons of neurons in the CNS [135]. Myelin is rich in cholesterol, which is synthesized *de novo* by glial cells, as cholesterol cannot be transported across the blood-brain barrier, and neurons have been thought to possess limited capacity for cholesterol synthesis [136, 137]. Increasing amounts of data also support the idea that glial cells, previously regarded as passive cells whose only function is to maintain neurons, may be required for the formation and maintenance of interneuronal synapses in the brain [138, 139]. Lipids, among them cholesterol, constitute key components in the efficient communication between neuronal and glial cells [133, 138-140]. Glial cells secrete apolipoprotein E (ApoE)-bound cholesterol, which is taken up by neurons by means of low-density lipoprotein (LDL) receptors and acts as a growth factor for neurons [138, 141, 142].

5.4.5 Regulatory factors in lipid biosynthesis

As Figure 5.1 shows, a large number of enzymes are involved in the synthesis of fatty acids and triglycerides. Many of these are primarily regulated at the transcriptional level, meaning that transcription of the genes encoding them is regulated in a coordinated manner [143]. The sterol regulatory element binding proteins (SREBPs) are transcription factors involved in numerous aspects of fatty acid, triglyceride, and cholesterol synthesis, and are frequently designated "master" transcription factors in lipogenesis, as they hold key positions in the coordinated transcription of lipogenic genes. Two main SREBP proteins, SREBP1 and SRBEP2, are encoded by two distinct genes. The SREBPF1 gene encodes two isoforms, SREBP1a and SREBP1c, of which SREBP1c is the isoform most extensively expressed in liver and adipose tissues in mice, while SREBP1a is primarily found in cultured cells [144]. SREBP1c is a main regulator of genes encoding enzymes involved in fatty acid and triglyceride metabolism, e.g. the genes encoding ACC1, FASN, SCD1, and GPAM [145-147]. SREBP2, encoded by SREBF2, controls the transcription of enzymes synthesizing sterols, including the rate-limiting HMGCR as well as HMGCS and several of the enzymes catalyzing later steps in the cholesterol biosynthesis pathway [147].

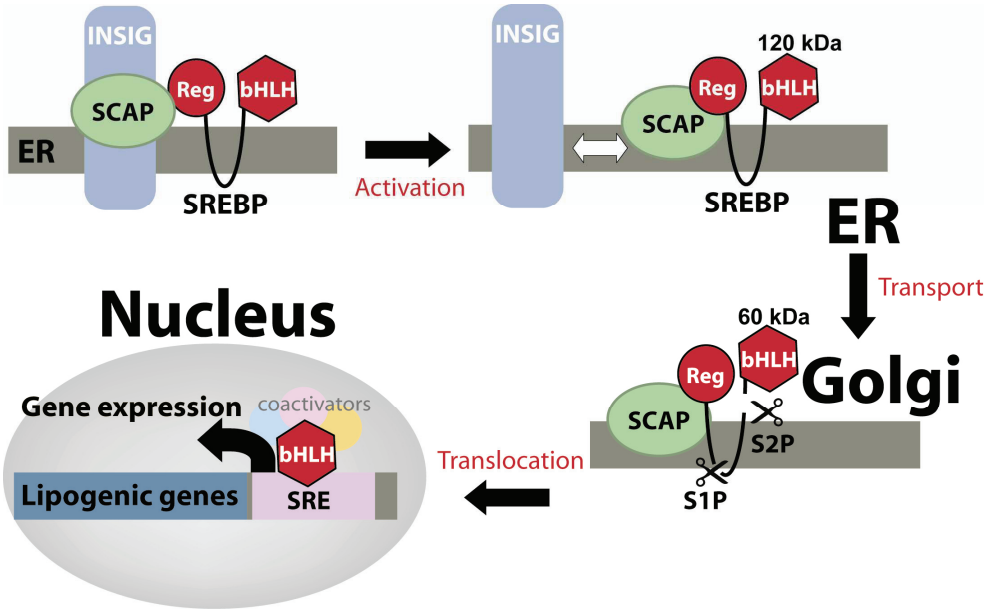

Figure 5.2 SREBP and its activation. SREBP, constisting of one regulatory domain and one domain containing a basic-helix-loop-helix leucine zipper (bHLH-Zip) protein [148]. The inactive form of SBERP forms a complex with SREBP cleavage activating protein (SCAP), a lipid sensor. Insulin-induced gene (Insig) immobilizes the SREBP-SCAP complex in the ER when lipid levels are high. Upon cholesterol depletion, SREBP-SCAP is transported to the Golgi apparatus, where the bHLH-Zip domain is released, translocating to the nucleus to initiate transcription of its target genes. SREBP1 is regulated by numerous nutritional factors (e.g. carbohydrates) [149], while SREBP2 is primarily regulated by cholesterol levels. Illustration by Johan Fernø.

The SREBP proteins reside in the endoplasmatic reticulum (ER) membrane as inactive precursor proteins of 120-130 kDa. Intracellular sterol depletion or other alterations in the cell's nutritional status result in translocation of the inactive SREBP protein to the Golgi apparatus, where proteolytic cleavage produces an active (nuclear) form of 60-70 kDa (Figure 5.2) [147]. A large number of lipogenic gene promoters contain sterol regulatory elements (SRE) or an E-box motif with affinity for cleaved SREBPs. As a key regulator of anabolic processes, SREBPs are activated in states of energy surplus, such as increased energy intake (in the form of

carbohydrates or lipids). Both saturated fatty acids and insulin promotes SREBP1c-mediated lipogenesis [147, 150, 151].

5.4.6 Mechanisms of fatty acid oxidation

When energy mobilization is required, free fatty acids may be released from triglycerides by lipolysis, and oxidised in the mitochondria in a process releasing ATP [128]. If energy reserves are depleted, free fatty acids are transported from white adipose tissues to skeletal muscle, heart, and liver, for oxidation [152]. Oxidation takes place in the mitochondrial matrix. Fatty acids, "activated" through linkage to CoA yielding fatty acyl-CoA, are linked to carnitine by carnitine palmitoyltransferase 1 (CPT1) before being transported across the outer mitochondrial membrane (Figure 5.1). In the mitochondrial matrix, the fatty acyl group is transferred from carnitine to a matrix-specific pool of CoA. The transient linkage to carnitine (carnitine shuttle) represents the rate-limiting steps of fatty acid oxidation. In the matrix, fatty acyl substrates are oxidised in a four-step process. Oxidation of one palmitoyl molecule (C16:0), which is broken down to 8 acetyl-CoA molecules, yields 28 ATP units (in addition, acetyl-CoA oxidation through the citric acid cycle yields further ATP) [128]. Organelles other than mitochondria, namely peroxisomes, may also be the site of fatty acid β oxidation, catalyzed by different enzymes than those found in mitochondria. In particular, the enzyme acyl-CoA oxidase 1 (Acox1), catalyzing the first step in peroxisomal β oxidation, is important.

5.4.7 Regulation of fatty acid oxidation and lipid storage

Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors sensing lipid levels and regulating a wide array of responses to altered lipid load. Three PPAR isoforms - PPARα, PPARδ, and PPARγ - are recognized, all transcription factors with a large number of target genes [152]. PPARα, highly expressed in the liver, induces β-oxidation in times of reduced energy access, e.g. in the fasting state. PPARγ is predominantly expressed in adipose tissues, and activates pathways facilitating lipid

storage through biosynthesis and adipocyte differentiation. Increased lipid storage capacity resulting from PPARγ activation is thought to be an important mechanism of action for the pharmacological PPARγ agonists thiazolidinediones (TZDs), presently used as insulin-sensitizing agents in patients [152].

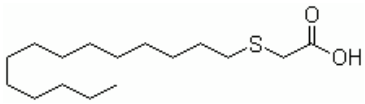

Figure 5.3 The structural formula of TTA.

5.4.8 Tetradecylthioacetic acid (TTA)

Tetradecylthioacetic acid (TTA) is an artificially synthesized fatty acid where the 3rd carbon atom is replaced with a sulphur atom, producing a non-oxidizable fatty acid derivative (CH_3-$(CH_2)_{13}$-S-CH_2-COOH). Acting as an agonist for all PPAR subspecies, with most potent effects on PPARα, TTA nevertheless induces the mitochondrial β-oxidative apparatus, resulting in increased mitochondrial oxidation of naturally occurring substrates for β-oxidation [153]. In male rats, TTA has been shown to prevent adiposity and insulin resistance induced by high-fat diet, decreasing plasma triacylglycerol and free fatty acid levels [153, 154]. Fibrates, another type of PPARα agonists used clinically in the management of hypertriglyceridemia [152], have also been shown to improve insulin sensitivity in rodents [155-157]. Small clinical trials have indicated metabolically beneficial effects of TTA in patients [158], and TTA was therefore included in our rat experiments.

6. Aims of the study

The overall aim of this study was to identify new molecular mechanisms underlying metabolic adverse effects of antipsychotic drugs, and to confirm their relevance by means of a rat model.

Specific aims:

- To identify differential metabolic effects of different antipsychotic drugs in various cultured cell types modelling cell populations in the CNS
- To clarify the role of hyperphagia in antipsychotic-induced weight gain, in a rat model
- To examine the possible uncoupling of weight gain from alterations in lipid metabolism in rat exposed to antipsychotic drugs
- To explore the use of a non-invasive imaging technique (MRI) for quantification of antipsychotic-induced adiposity in rat
- To examine the development of food intake, weight gain and lipogenic alterations in long-term antipsychotic treatment in rat
- To investigate the lipid-lowering, modified fatty acid TTA as a potential pharmacological intervention strategy for metabolic adverse effects

7. Summary of results

Paper I

The experiments in Paper I were based on results from our initial microarray studies in antipsychotic-treated, cultured cells [159, 160]. Examining the effects of a number of antipsychotics on lipogenic gene expression in human neuron-like and glial-like cell types we found that clozapine, one of the two most metabolically potent antipsychotics in humans, and chlorpromazine, the "prototype" first-generation antipsychotic, activated the transcription of lipogenic genes with most pronounced effects in glial-like cells. Lipogenic activation was mediated by the SREBP transcription factor, a master regulator of lipogenesis.

Paper II

Rats were treated with olanzapine or aripiprazole for 13 days. As expected, olanzapine-treated rats increased their food intake and gained weight in the form of increased adipose tissue mass, demonstrated by weighing and MRI-based quantification of adipose tissue. Aripiprazole, included as a negative control due to its clinical status as metabolically neutral, induced a similar pattern. In an olanzapine-treated group of rats with limited food access, weight gain was absent. However, serum triglyceride levels were increased in both olanzapine treatment groups, as was lipogenic gene expression in visceral adipose tissue. Aripiprazole-treated rats did not develop these features. We concluded that factors other than weight gain may significantly contribute to antipsychotic-induced metabolic derangements.

Paper III

Seeking to elaborate our findings from Paper II, we extended the treatment period to 8 weeks, included two new antipsychotics and slightly lower drug doses. The weight gain-inducing effects of olanzapine wore off ~ 3 weeks into the experiment, while treatment with clozapine failed to induce weight gain. The modified fatty acid TTA potentiated weight gain both in combination with olanzapine and clozapine, with concomitant reduction in plasma and liver lipid levels. The lipid-lowering effects of TTA were accompanied by substantial increase in the transcription and enzymatic activity of the key oxidative enzymes ACOX1 and CPT2 in the liver, as well as reduced transcription of the rate-limiting enzyme in cholesterol, HMGCR. While calling the relevance of the female rat model into question, the results supported the concept of weight-lipid uncoupling.

8. Discussion

8.1. Methodological aspects

8.1.1 Cell culture

In paper I, we examined potential lipogenic effects of antipsychotic drugs in five different cell cultures. The use of cell cultures is extremely widespread in biological research. Among the numerous advantages of using cultured cells are the easily controlled environment, e.g. availability of nutrients, and flexible experimental setups, e.g. regarding drug doses. Furthermore, working with cell culture circumvents most ethical considerations. However, even though cultured cells interact, traditional cell cultures represent a considerable oversimplification, since interaction between different cell types usually present in an organ, as well as tissue-tissue interaction, are absent. In addition, cells are usually transformed, usually malignantly, in order to enable division and growth in culture. In many instances, such "non-physiological" conditions are necessary in order to discriminate relevant molecular processes from feedback responses and other compensatory events normally present in a complete organism. Using two neuron-like (HCN2 and SH-SY5Y) and two glial-like (GaMg and CCF-STTG1) human cell lines, as well as one hippocampal primary culture from rat (R-Hi-501), we found corresponding upregulation of SREBP target genes in all cell types, but with minor effects in cells derived from neurons. The common pattern observed across cell lines indicated that antipsychotic-induced SREBP activation observed in GaMg cells in our previously published article [159] is not limited to one type of cultured cells, and could represent a generalized drug effect.

8.1.2 RealTime PCR

In order to quantify expression levels of potentially relevant genes in both cell culture and in rat tissues, we have extensively used RealTime PCR. This method is based on the concept of a fluorescent probe or a DNA-binding dye being released as mRNA is replicated throughout 35-40 PCR cycles. Continuous quantification of released probe or dye permits "real-time" quantification of mRNA levels [161]. The PCR cycle during which the level of probe or dye is first detected at a higher level than the sample background represents the gene's cycle threshold (Ct) value [161]. The RealTimePCR method is highly sensitive to alterations in gene expression across a wide range of expression levels, i.e. even for genes with particularly high or low expression levels in a certain tissue [161]. We used SYBR® Green, a DNA-binding dye, and primers designed in-house, for all RealTime reactions. For analysis of results, we used the comparative Ct method ($2^{-\Delta\Delta}$Ct method), with normalization towards one or more endogenous control genes. Here, the relative difference in gene expression between antipsychotic-treated samples and vehicle-treated samples, with the latter used as calibrator, was calculated based on the genes' Ct values. This method assumes similar replication efficiencies between genes, controlled by means of serial dilutions, and is more reliable if PCR products are kept below 150 bp. We therefore designed utilized primers such that the PCR product size was kept below this size for all genes.

In order to control for differences in RNA input in the reverse transcription stage, Ct values for all target genes were normalised to Ct values for genes thought to be stably expressed in target tissues (i.e. housekeeping genes). Selection of endogenous control genes is a major challenge when using RealTime PCR, and the expression of several housekeeping genes have been shown to be affected by drug exposure in cell culture [162].

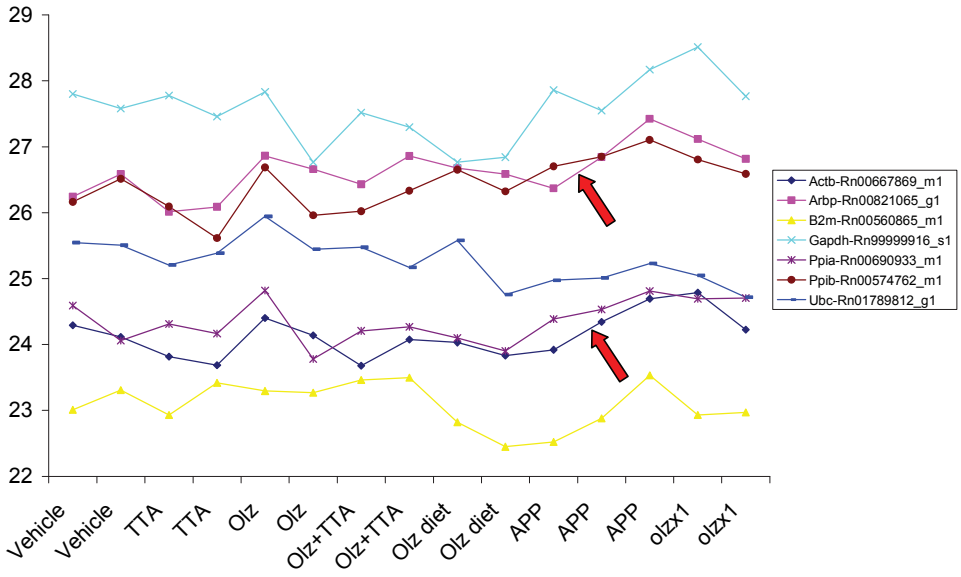

Figure 8.1 Ct values (Y axis) for 7 potential endogenous control genes in white adipose tissue from rats exposed to different pharmacological treatments. Each data point represents one sample. Samples were taken from rats exposed to different pharmacological treatments. Each coloured curve represents one potential reference gene. Arrows signify the two endogenous control genes used in our rat experiments.

Preparing to analyze tissues not previously examined in our lab (particularly white adipose tissues), we ran selected samples on predesigned panels (low density arrays, LDA; Applied Biosystems) containing several pre-selected endogenous control genes commonly used (Figure 8.1; unpublished data), in order to select stably expressed housekeeping genes. Based on LDA results, candidate genes for further use as endogenous controls were chosen and further evaluated using different RealTime assays. The reference gene used in Paper I, ribosomal protein, large, P0 (Rplp0; designated P0 in Paper I-III) in addition to the commonly used β-actin, were selected for use in further analyses, and these two reference genes were run in each new batch of cDNA in the rat experiments. In order to detect potential systematic treatment effects on endogenous control genes, Ct values in vehicle- and antipsychotic-treated rats were habitually compared for all examined genes during analysis of RealTime

PCR data. Results from several biological replicates have confirmed our initial patterns of transcriptional regulation by antipsychotic agents, increasing the validity of results. Furthermore, several key findings were validated at the protein level using Western blots.

8.1.3 MRI-based quantification of adipose tissue volume

Paper II includes tissue imaging data collected using a 7T MRI scanner. Olanzapine- and aripiprazole-exposed rats included in a 2-week experiment underwent MRI scanning prior to the initiation of treatment, and by the end of the treatment period. The protocol used for image analysis was developed by in-house collaborators. In the attempt to extract quantitative data (adipose tissue volumes) from MRI images, two major challenges deserve mention. Firstly, in order to quantify alterations in white adipose tissue mass between two time points, one needs reliable anatomical landmarks. Scanning the entire animal was not an option, as this would require an unreasonable amount of time (considering both time spent in anaesthesia and experimental logistics). As MRI is not a sensitive method for imaging of skeletal parts, the early idea of using lumbar vertebrae as landmarks was dismissed. Instead, we chose to use the easily visible kidneys. A second challenge was the distinction between white adipose tissue and artefacts, particularly in the intestine. The segmentation protocol developed to distinguish and quantify adipose tissue yielded a relevant impression of increased adipose tissue volume in olanzapine-treated rats, but numerical estimates were not significantly correlated with dissected adipose tissue mass (Paper II). The reasons behind this discrepancy remain unclear. In further developing MRI acquisition and analysis, a natural first step would be to increase the anatomical area examined, e.g. by scanning the entire abdominal area. Alternative methods (e.g. dual-energy X-ray absorptiometry [DXA] scans) are available for quantification of total adipose tissue mass in rodents (reviewed in [163]). MRI images permit the distinction of different adipose depots (visceral, subcutaneous), and as demonstrated by us, rodents could be examined at several time points during the same

experiment, with no significant mortality among examined rats. These are among the advantages that should encourage further use and development of MRI protocols and analysis tools in studies centred on metabolic adverse effects.

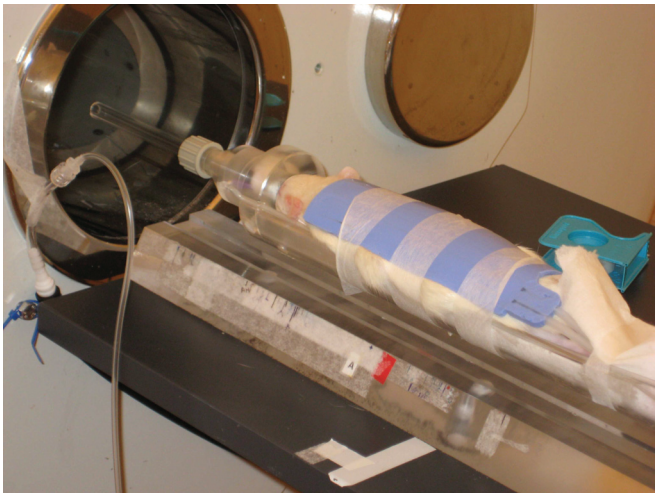

Figure 8.2. Deeply anaesthetized rat mounted for MRI examination, covered by a heating mat (blue). Photograph by Silje Skrede.

8.1.4 Selecting a drug vehicle

Most antipsychotic agents are close to insoluble in water, but readily dissolved in DMSO or alcohol. Concerns over toxicity, vehicle-induced biochemical effects confounding results, and palatability issues led us to search for an appropriate vehicle for use in rat studies involving oral administration of antipsychotics. Eventually, based on our own experiments and relevant literature, 4% carboxymethyl cellulose (CMC) was chosen [114, 117]. Antipsychotics were suspended, not dissolved, in the CMC solution. As the drugs rapidly precipitated, frequent resuspension was necessary in order to maintain the correct drug concentration during administration to rats, potentially introducing dosing inaccuracy. Measurements of serum drug levels

showed modest variation across antipsychotic-treated rats at the time of sacrifice, indicating adequate dosing of drug suspended in CMC.

8.2 Modelling metabolic adverse effects in rat

8.2.1 Divergent findings in human and rat

As mentioned in the Introduction (see Table 5.5), several aspects of metabolic adverse effects diverge in rodent and human, primarily considering gender differences (observed in rat, not convincingly demonstrated in humans) and dysmetabolic potencies between different antipsychotic agents. In particular, the near absence of metabolic alterations in clozapine-treated rats remains puzzling.

The question of gender specificity raises the question of whether endocrine factors may play a more significant role than has been demonstrated thus far. Antipsychotics, as recognized dopamine receptor antagonists, are known to increase prolactin levels in patients [164, 165]. Olanzapine has been demonstrated to increase serum prolactin levels both in female [117, 124] and in male rat [105, 166], indicating that hyperprolactinemia *per se* cannot provide a straightforward explanation for the gender pattern observed in rat. Oestrogens are closely linked with distribution of adipose tissue as well as several other aspects of energy metabolism [167], and may be relevant to the gender differences observed in rat. One study found unaltered serum oestradiol levels in female, olanzapine-treated rats [124]. In ovariectomized rats, with negligible oestradiol levels, olanzapine has still been demonstrated to induce food intake and weight gain [168]. In patients, olanzapine treatment had no effect on oestradiol levels, neither in males [169] nor in females [170]. Even though other endocrine factors could be relevant, there are no obvious candidates in the relatively limited number of studies examining hormonal effects of antipsychotics with high dysmetabolic potential [171].

During the last few years, a quite differentiated picture of antipsychotic-induced alterations in rodents has emerged. For instance, increased adiposity in spite of constant body weight after subchronic olanzapine treatment of male rats has been described in several studies [105, 106]. Through these, and our subchronic experiments demonstrating olanzapine-induced serum triglyceride elevation, an increased number of shared features in human and rodent may emerge, adding to the relevance of rodent models. Nevertheless, in further development of animal models for antipsychotic-induced metabolic adverse effects, several important challenges remain.

8.2.2 Challenge I: pharmacokinetics of antipsychotics in rat

In rat, metabolism of antipsychotic agents differs significantly from human metabolism of these drugs. For instance, the half-life ($t_{1/2}$) of clozapine in rat serum is 1.5 hours, while the average $t_{1/2}$ is 10 hours in human [172, 173]. Similar differences are found for olanzapine, with a $t_{1/2}$ of 2.5-3 hours in rat, compared to an average of 30 hours in human [174-176]. The rapid metabolism of antipsychotics in rat complicates the selection of appropriate drug doses in rat experiments, as serum drug concentrations rapidly reach negligible levels.

8.2.3 Challenge II: dosing of antipsychotics in rats

Defining a "correct" dose for antipsychotics in rats based on clinical observation is difficult. A commonly used approach when selecting doses for rat experiment is direct transfer of doses used in patients. In many countries, the maximum olanzapine dose approved for use in patients is 20 mg, i.e. ~0.3 mg/kg in a person weighing 70 kg [177]. As for clozapine, patients may receive 600 mg, i.e. ~8.6 mg/kg [177]. In rats, these doses are too low to induce weight gain [108, 109, 111, 178]. Based on the significant differences in human and rodent drug metabolism, a dosing strategy based on the percentage of D_2 receptor occupancy in the CNS has been suggested [179]. In order to achieve D_2

occupancy comparable with that observed in humans (65-80% for olanzapine and 45-65% for clozapine) after a single dose of antipsychotic agents in rat, olanzapine doses in the range of 1-2 mg/kg and clozapine doses of 5-15 mg/kg have been recommended [179]. As mentioned in the Introduction, raising clozapine doses in rats to this level has so far not yielded weight gain. In fact, in one subchronic study rats received 30 mg/kg clozapine, with absence of weight gain [180]. Notably, in male rats receiving 20 or 40 mg/kg clozapine, D_2 occupancy in the CNS was far below the levels observed in humans at clinically relevant doses; body weight was not measured [181]. High doses (olanzapine: ~ 20 mg/kg; clozapine: 10-20 mg/kg?) may cause sedation [108, 182], and this fact has influenced the doses selected for studies in rodents. Some studies, however, reported absence of sedation in rats in the 20-40 mg/kg clozapine dose interval [180, 181]. Thus, underdosing may represent an explanation for the lack of weight gain in clozapine-treated rats.

A second dosing-related issue is drug tolerability. In patients, weight gain is typically considered to continue for approximately one year [183, 184] before body weight reaches a plateau, although this issue is debated (reviewed in [100, 185]). Weight gain in rats treated with low to moderate doses of olanzapine (1-6 mg/kg) seems liable to dwindle during treatment, as indicated by our results, with dampened effects on weight gain after 2-3 weeks of treatment Papers II, III), although one group showed continued weight-inducing effect of olanzapine 1,5 mg/kg for 8 weeks [113]. A stepwise increase of olanzapine doses, from 4 to 20 mg/kg, potentiated weight gain compared to our results, but at 33 days, cumulative weight gain for rats treated with control substance and olanzapine (end dose 20 mg/kg) was nevertheless relatively similar [108]. Stepwise dose increase may, however, circumvent sedation, and should be considered in long-term rat experiments.

8.2.4 Challenge III: administration of antipsychotics to rats

Administration of antipsychotics to rats is commonly achieved by means of subcutaneous or intraperitoneal injections [124, 186], oral administration through gavage [102], through food [106] or through drinking water [105, 181]. As the number of daily injections is, for practical reasons, limited, and food/water intake is unevenly distributed throughout the light/dark phases, all these approaches are likely to result in fluctuating serum concentrations during a 24-hour period [181]. Several antipsychotic agents degrade when exposed to light, further complicating the approach of mixing drugs with food or drinking water. In order to ensure constant delivery of non-degraded antipsychotics, some researchers have utilized osmotic minipumps, with subcutaneous or intraperitoneal implantation of a syringe constantly delivering a fixed dose of dissolved drug. As pumps previously needed to be refilled frequently due to drug degradation, the use of osmotic minipumps could be more labour-intense than anticipated [187]. Implantation of syringes carries a risk for infection, and minipumps are subject to mechanical failure. In carefully controlled conditions, using light-protected minipumps, dissolved olanzapine has been demonstrated to remain stable for 42 days [188]. When successful, serum drug concentrations reached by means of minipumps are higher and more stable than those achieved through other means of administration [118, 188]. The use of minipumps, therefore, may be preferable to intermittent oral dosing or injections. Other potential strategies include pellets designed for subcutaneous implantation, followed by constant drug release, which are available, but have not been extensively used [189].

8.2.5 Challenge IV: the influence of diet

Standard rat chow used in laboratories is very high in carbohydrate (40-50%), with a low fat content (~5%). In male, olanzapine-treated rats and in female, clozapine-treated rats, the limited number of studies demonstrating weight gain included chow

with high fat content [110, 119, 190]. Some studies with very similar setups, however, have not yielded significant weight gain [106, 190, 191]. Finding the "ultimate" dietary composition may not be sufficient to achieve weight gain in male rats treated with olanzapine or in clozapine-treated rats, but is likely to represent one of several necessary conditions.

8.2.6 Steps towards increased reliability of rat models

The possibility remains that a certain constellation of drug dose, mode of administration, and dietary fat content will lead to successful replication of a more "human-like" pattern of antipsychotic-induced metabolic adverse effects than has previously been achieved. In two recently described experiments, male rats were fed high-fat chow and treated with 1.5-10 mg/kg olanzapine administered by minipumps; still, only marginal effects on body weight gain were observed, in spite of increased adipose tissue mass [188, 192]. Experiments including even higher antipsychotic doses than previously administered to rats may be a natural next step. Of course, one can ask whether the need to "fine-tune" several parameters will yield a sufficiently robust model - even if clozapine-induced weight gain is successfully modelled, the fact may remain that some as yet unrevealed property distinguishes metabolic effects of olanzapine in rat from that of clozapine.

8.4. Molecular mechanisms of metabolic adverse effects

8.4.1 Hyperphagia is the main cause of body weight gain

Weight gain may be caused by increased energy intake, reduced energy expenditure, or a combination of the two. In order to investigate the role of food intake in olanzapine-induced weight gain, we included olanzapine-treated, pair-fed treatment groups in our rat experiments. Pair-fed rats were offered an amount of chow corresponding with the average amount consumed by control animals during the preceding 24 hours, typically 15-16 grams per rat. Freely fed olanzapine-treated rats may consume 20-24 g of chow during 24 hours. We observed that olanzapine-treated rats with free access to chow rapidly gained weight, while pair-fed rats did not gain weight, or even gained less weight than control animals (Paper II). This led us to conclude that increased food intake (hyperphagia) is the main driving force behind the observed weight gain, in agreement with previous rat studies [88, 121] as well as clinical studies [193, 194]. Our MRI studies, coupled with dissection and weighing of adipose tissues, demonstrated that weight gain occurred primarily in the form of increased adipose tissue mass, also in agreement with previous results in rat (Table 5.5) [103, 124] and in patients [60, 61]. The pair-fed rats in our experiment did not show signs of increased adipose tissue mass. In an article not included in this thesis, our group has explored the possible mechanisms of antipsychotic-induced hyperphagia, demonstrating increased levels of appetite-stimulating neuropeptides in hypothalamus [195].

8.4.4 The role of energy expenditure in weight gain

In addition to increased energy intake, reduced energy expenditure may contribute to weight gain. As previously mentioned, sedation resulting in reduced physical activity is a recognized adverse effect of antipsychotic agents. We did not, however, observe

significant sedation in the experiments described in Paper II. If sedation contributed significantly to weight gain, the obese phenotype would have been expected to be present in the olanzapine pair-fed treatment group.

Rodents possess brown adipose tissue, which is able to convert surplus energy to heat through a process termed thermogenesis [196]. Reduced thermogenesis, measured as reduced protein levels of uncoupling protein 1 (UCP1), has been reported in pair-fed, olanzapine-treated rats which gained weight, in contrast to pair-fed rats in our experiments [197]. In a previously mentioned study, olanzapine treatment did not induce altered thermogenic rates in brown adipose tissue from rats with stable body weight, but increased adiposity [178]. Based on these conflicting findings, we examined the expression of UCP1, PPAGγ, and peroxisome proliferator activated receptor gamma coactivator 1α (PGC1α), important markers of thermogenesis, in brown adipose tissue [198]. Several of these markers were downregulated in both *ad libitum*-fed and pair-fed olanzapine groups, possibly signifying reduced thermogenesis. Although synergistic effects of hyperphagia and reduced thermogenesis in the olanzapine *ad libitum*-group cannot be ruled out, the lack of weight gain in the olanzapine pair-fed group demonstrated that reduced thermogenesis alone did not significantly contribute to weight gain in our experiments. In our 8-week experiment (Paper III), TTA monotherapy downregulated PGC1α and UCP1, while no weight gain was observed in this treatment group. In olanzapine-TTA treated rats, PGC1α was also downregulated, possibly contributing to the weight gain observed in this treatment group.

Adult humans were formerly thought to lack brown adipose tissue. In recent years, however, the presence of brown adipose tissue in adults has been demonstrated by means of PET scans [199-201]. Brown adipose tissue may play a significant role in human metabolism, and should not be ignored when examining energy balance [201].

8.4.5 The role of fatty acid oxidation in antipsychotic-induced metabolic adverse effects

Reduced capacity for fatty oxidation could, theoretically, induce energy surplus and thus obesity. Examining the expression of ACOX1, CPTs, and PPARs in liver and white adipose tissues from subchronically treated rats (Paper II) or from chronically treated rats (Paper III), we found no evidence for reduced oxidative capacity induced by olanzapine or clozapine. The lipid-lowering effects of TTA, on the other hand, were likely related to the sharp increase observed in hepatic ACOX1 and CPT1 transcription and activity.

8.4.6 "Uncoupling" of body weight and serum lipid levels

Hypertriglyceridemia is known to correlate with obesity, in particular with the amount of visceral adipose tissue. However, the existence of both "obese, but metabolically healthy" and "metabolically obese" populations is now recognized [202]. The latter group is characterized by normal BMI, but still present with dyslipidemia and reduced insulin sensitivity [203]. In paper II, through the introduction of pair-fed treatment groups, we found that olanzapine-treated rats developed elevated levels of serum triglycerides without concomitant adiposity. These results were further supported by results in paper III, in which olanzapine-TTA treated rats gained weight, while nevertheless developing lowered serum and liver levels of cholesterol and triglycerides, resulting in lipid profiles not expected in rats with this degree of obesity.

Interestingly, early clinical findings, both of chlorpromazine-induced increase in serum cholesterol levels and olanzapine-induced increase in serum triglyceride levels, indicated that serum lipid levels in patients were not simply raised secondary to weight gain [52, 65]. Several recent clinical studies have also presented evidence for increased serum triglycerides independent of weight gain [204-206]. In the CAFE study, patients treated with quetiapine experienced the least increase in weight gain; in spite

of this, increase in serum triglycerides and total cholesterol were most pronounced in this treatment group [207]. Of note, in a "drug switching" study in which patients changed from olanzapine to aripiprazole treatment, a significant reduction in serum triglycerides occurred rapidly after the switch, while weight loss occurred more gradually, indicating that triglyceride levels did not simply decrease in parallel with body weight [208]. To our knowledge, no animal studies other than our own have investigated the potential uncoupling between weight gain and increased serum lipids.

8.4.7 Antipsychotic-induced, SREBP-mediated lipogenic activation in cultured cells

In addition to our papers describing antipsychotic-induced SREBP activation in GaMg cells, our research group published a work demonstrating similar effects in human liver cell lines (THLE-3 and HepG2) [160]. Several studies in cell culture have subsequently supported our finding of antipsychotic-induced SREBP activation in cells, including adipocyte cultures and primary hepatocytes [209-212]. In paper I, we demonstrated that antipsychotics induce both SREBP1 and SREBP2 target genes. We showed increased proteolytic cleavage of SREBP2, i.e. activation at the protein level, and upregulation of the genes encoding the SREBP1a and SREBP2. The exact nature of the interaction between antipsychotic agents and the SREBP system remains unclear, but may be related to hampered intracellular cholesterol transport through direct interaction between drugs and intracellular membranes [213, 214]. Chlorpromazine, which was demonstrated by us to induce cholesterol biosynthesis genes in GaMg cells, is a tricyclic cationic amphipathic drug with both lipophilic and hydrophilic chemical properties [215]. Several antipsychotics, among them clozapine, also possess a tricyclic structure, and have been demonstrated to directly interact with the cholesterol biosynthesis pathway [212, 215-217]. Possibly, reduced ER cholesterol causes "imaginary" lack of intracellular cholesterol, inducing SREBP translocation, maturation and thus target gene transcription. Recently, an antipsychotic-induced ER stress response (unfolded protein response) has been suggested to contribute to SREBP activation,

offering a potential explanation for the activation of both SREBP isoforms, which are differentially regulated in the physiological setting [218].

8.4.8 Antipsychotic-induced lipogenic activation in rodents and humans

Prior to our subchronic studies, we performed acute experiments on clozapine and olanzapine treatment in rats. We found short-term lipogenic effects, most notably significant hepatic lipid accumulation, accompanied by an early transcriptional activation followed by sustained downregulation of lipogenic genes in liver and adipose tissues [219, 220]. These observations illustrate the presence of potent negative feedback mechanisms complicating interpretation of direct pharmacological effects of antipsychotics in rat. In our two-week studies with moderate olanzapine doses (Paper II), feedback mechanisms were partly circumvented through the reduction of drug doses and repeated exposure, and we demonstrated weight-independent upregulation of several SREBP1c target genes in liver and in visceral adipose tissue. Corresponding upregulation of *Fasn* has previously been demonstrated in adipocytes from male, olanzapine-treated rats that did not gain significantly more weight than control animals during 5 weeks of treatment, but developed increased adipose tissue mass [107]. Furthermore, in our subchronic study (Paper II), we found signs of SREBP1c activation in the liver, both at the transcriptional and at the protein level. In agreement with other studies, serum levels of monodesaturated fatty acids were increased, indicating elevated desaturase activity [221, 222]. Upregulation of Scd1 transcription is notable, as this desaturase has been suggested to represent a key branch point in lipid biosynthesis, directing lipids towards triglyceride or cholesterol ester formation (i.e., lipid storage) [223].

In visceral adipose tissue, in spite of upregulation of several recognized SREBP1c target genes, *Srebpf1* transcription or SREBP1c activation at the protein level were absent. In fact, there are indications that in adipocytes and adipose tissues, SREBP1c excerts less profound effects on the transcription of its classic target genes than in the liver [224-227]. Other, as of yet unrevealed, mechanisms may contribute to olanzapine-

induced lipogenic activation in adipose tissues. As lipogenic upregulation was present in olanzapine pair-fed rats, this induction is likely to represent a pharmacological effect of olanzapine, not a consequence of hyperphagia and obesity.

In conclusion, we found partial overlap of results in cell culture and animal models, underlining the complexity of lipid metabolism in the "real-life" setting. SREBP-mediated lipogenesis may be relevant for dyslipidemic adverse effects of antipsychotics, independent of hyperphagia and weight gain. Further experiments are required in order to examine potential subchronic effects of clozapine on lipogenesis, the relationship between lipogenic activation in metabolic tissues and the elevation of serum triglycerides, to identify the factors mediating lipogenic effects of antipsychotics in adipose tissues, and to perform relevant clinical studies. Interestingly, a clinical pilot study demonstrated elevated transcription of the genes encoding FASN and SDC1 in blood cells from olanzapine-treated patients [228].

8.5. Clinical aspects related to lipogenic activation by antipsychotics

8.5.1 Do metabolically potent antipsychotic agents have superior clinical efficiacy?

Whether some antipsychotic agents are more efficient than others in relieving either positive or negative symptoms of schizophrenia - in particular, whether second-generation antipsychotics are more efficient than first-generation drugs - has been extensively debated [229-237]. Results from several large clinical studies, among them the CATIE and CUtLASS studies, indicate that despite trends towards superior efficacy of olanzapine on certain outcomes (primarily time to treatment discontinuation), atypical antipsychotics are not convincingly superior to first-generation antipsychotics in terms of overall clinical efficiency or quality of life scores [177, 233, 238, 239]. As

previously mentioned, several reports have documented the superior efficacy of clozapine in treatment-resistant schizophrenia [42, 177]. In fact, a large population-based Finnish study demonstrated significantly reduced mortality in patients treated with clozapine compared to patients treated with other antipsychotics, in spite of clozapine's metabolic risk profile [240]; this study subsequently received criticism for strongly confounded analyses [241].

Comparisons of typical and atypical antipsychotics are complicated, among other issues, by differences in dosing and adverse effect profiles. For instance, motor effects of typical antipsychotics could result in the impression that patients suffer from more severe negative symptoms [242]. Potential interests of the pharmaceutics industry must also be kept in mind when evaluating treatment effects [243]. In general, issues such as adverse effect profile, former response to medication, patient satisfaction, cost-effectiveness, and clinicians' former experiences are significant issues when patients with suspected schizophrenia are assigned to a certain pharmacological treatment [177, 231, 244]. Naturalistic, non-sponsored studies are required to further facilitate evidence-based choice of treatment of psychosis [245].

8.5.2 Are the differences in dysmetabolic potency between different antipsychotic agents as substantial as formerly thought?

As mentioned above, adverse effect profile constitutes an important consideration when selecting an antipsychotic, particularly for patients experiencing their first episode of symptoms. Therefore, a balanced image of adverse effect profiles is required. In paper II, as expected, we found a significant stimulatory effect of olanzapine on food intake and weight gain in female rats. Aripiprazole, believed to be metabolically neutral in humans, was included as a negative control, and both in our subchronic and in our chronic (8-week) experiment (Paper III), aripiprazole showed hyperphagic and weight-inducing potential. One former experiment in female rat

resulted in aripiprazole-induced weight gain (aripiprazole dose 4-8 mg/kg) [103], while a long-term experiment yielded no weight gain (aripiprazole dose 2.25 mg/kg) [113]. This could indicate that body weight in female rats is more easily affected by pharmacological treatment than body weight in human subjects. On the other hand, could these results be transferred back to the clinical setting, helping to define a more differentiated picture of the dysmetabolic potential of individual antipsychotics? It is quite obvious that in patients, some antipsychotics - particularly clozapine and olanzapine - induce more potent weight gain, dyslipidemia and insulin resistance than other agents. Furthermore, the switching of treatment from olanzapine, quetiapine, or risperidone to aripiprazole in patients led to significant improvemens in body weight and lipid parameters [74, 208]. However, it is obligatory to keep in mind the fact that a majority of clinical data is obtained through studies involving previously medicated patients, which may significantly influence results. The value of data collected in previously unmedicated patients is gaining increasing attention [246]. For instance, quetiapine and risperidone, have been shown to cause significant weight gain (≥7% of pre-tratment body weight) after 1 year of treatment in 50% of treatment-naïve patients receiving quetiapine, and in 58% of treatment-naïve patients receiving risperidone [62, 207]. Similarly, aripirazole, quetiapine and risperidone all caused weight gain in adolescent patients [247]. As agents such as quetiapine and aripiprazole have been in clinical use for an increasing number of years, more data concerning their metabolic adverse effects will accumulate, and results from rat experiments may turn out be more relevant than they presently appear.

8.5.3 Are clinical improvement and metabolic adverse effects correlated, independent of antipsychotic agent?

As discussed in paragraph 8.5.1, no sound conclusion has been reached regarding superior efficacy of individual antipsychotics. The idea of a link between symptom relief and metabolic adverse effects has been a recurrent issue for decades of research on antipsychotics. In 1967, an article concerning patients treated with chlorpromazine

stated that serum cholesterol levels were positively correlated with clinical improvement [52]. A number of studies, both in patients treated with olanzapine and in clozapine-treated patients, have pointed out a similar link between metabolic adverse effects, commonly weight gain, and improvement of positive or negative schizophrenic symptoms [205, 248-251]. One study found a relation between therapeutic response and weight gain both in olanzapine and haloperidol treatment groups, most pronounced in olanzapine-treated patients [98]. Another study, which has received criticism for prophylactically administering the anticholinergic drug to benztropine to all patients receiving haloperidol, reported positive correlation of serum cholesterol and improved cognition in schizophrenic patients across clozapine, olanzapine and haloperidol treatment groups [252].

Findings such as these are highly interesting considering that antipsychotics recognized to have the highest incidence of weight gain, hyperlipidemia and glucose dysregulation remain widely used [85]. However, some studies have failed to detect correlation of clinical state and metabolic adverse effects; for instance, analyses of data from the CATIE study resulted in drug-independent association between increased BMI and improvement, but the effect size was deemed too subtle to be clinically significant [205, 253]. Furthermore, lack of correlation between clinical improvement and weight gain has been reported in clozapine-treated patients [254, 255]. A recent study in hospitalized psychotic patients found that quetiapine, which is less metabolically potent than olanzapine, was more efficient than olanzapine in reducing several treatment outcomes [245]. Several issues complicate correlation analyses of metabolic adverse effects and clinical improvement. For instance, regain of self-care (including increase in food intake), could precede weight gain. No mechanistic link has yet been suggested between clinical response and metabolic adverse effects, but antipsychotic-induced lipogenesis is highly interesting in this context.

8.5.4 Lipogenesis as a possible therapeutic mechanism of action

As mentioned in the Introduction, demyelination (decreased volume or disrupted function of white matter) is presently thought to play a significant role in the development of schizophrenia, and may be of particular importance with regard to negative symptoms [18, 256-258]. Oligodendrocytes, the cells primarily responsible for myelin production, synthesize cholesterol, an essential component of myelin, as cholesterol is not imported to the CNS [133, 137]. Interestingly, a physiological role of the SREBP transcription factors in the CNS is emerging (reviewed in [259]). Lipid synthesis, controlled by both SREBP1 and SREBP2, in different subtypes of glial cells is thought to be important both in myelination and various aspects of neuronal development, such as synaptic plasticity. For instance, SREBP1 and SCD1 activation, correlated with oleic acid synthesis in astrocytes, may be involved in neuronal growth and differentiation [259]. Thus, alterations in lipid metabolism in the CNS may be highly relevant in light of the demyelination, as well as other aspects of neuronal dysfunction, observed in patients with schizophrenia. Indeed, myelin-related genes have been shown to be downregulated in prefrontal cortex from patients [260, 261]. In paper I, we demonstrated that SREBP2-activating effects of antipsychotics were more potent in glial-derived cells than in cells derived from neurons. A similar study on antidepressant drugs yielded comparable results [262]. Consequently, our results demonstrating antipsychotic-induced induction of lipid metabolism may be relevant both to the clinical and adverse effects of these drugs. In fact, the potential effects of antipsychotic agents on myelination have been investigated in a rodent model for demyelination, where the copper chelator cuprizone was used to pharmacologically induce demyelination. In rats treated with cuprizone, which developed phenotypic features reminiscent of negative symptoms of schizophrenia, downregulation of oligodendrocyte markers was demonstrated in the prefrontal cortex [263]. In another experiment on mice, clozapine and quetiapine was found to prevent loss of myelin induced by cuprizone [264]. In male patients with schizophrenia, treatment with risperidone led to increased myelination, as quantified by means of MRI [265, 266]. A

recent review by the first author of the latter article states that "[…] widely used psychotropic treatments have under-appreciated CNS metabolic and neurotransmitter effects on myelination, its plasticity, and repair that may substantially contribute to their mechanisms of action" [267]. The possible relation between clinical efficacy and metabolic adverse effects is intriguing, and the results discussed above underline the fact that avoiding all use of antipsychotics with metabolically unfavourable adverse profiles does not provide an easy solution to the huge clinical challenge represented by metabolic adverse effects.

8.5.5 Potential intervention strategies in patients with antipsychotic-induced dysmetabolism

88% of patients with dyslipidemia identified in the CATIE study population received no lipid-lowering treatment [79]. In general, secondary prevention of metabolic complications of antipsychotic treatment appears to have received little attention. Clinical studies have suggested that adjunctive treatment with metformin may have beneficial effect on insulin sensitivity and body weight [268-270]. Statins, i.e. inhibitors of HMGCR, have been demonstrated as efficient in antipsychotic-induced dyslipidemia [271]. Notably, metformin indirectly inhibits HMGCR through the activation of AMPK [272], meaning that both classes of agents regarded as candidates for efficient intervention act on the rate-limiting step in cholesterol synthesis.

Prior to pharmacological intervention strategies, information regarding hyperphagia and the risk of developing metabolic adverse effects should be given; patients may be surprisingly accessible to educational measures [273]. A few relatively small studies have addressed the effect of healthy dietary habits and exercise, and found promising effects [274-276]. Combining behavioural and pharmacological intervention strategies could probably reduce the risk for cardiovascular disease in patients receiving antipsychotic agents, but it is difficult to imagine that other measures than newly developed, clinically efficient drugs with negligible adverse effects will reduce risk levels to those found in the general population.

9. Concluding remarks

In selecting the correct treatment for psychotic, delusional or cognitively impaired patients, a substantial responsibility rests on the carer. Patient compliance is more crucial and challenging in schizophrenia than in many somatic conditions, and facilitating compliance greatly reduces the risk of relapse. Risk-benefit considerations must rely on solid evidence for clinical efficiency and adverse effects. A survey of the massive body of literature published regarding clinical effects of antipsychotic agents leaves no definite conclusion with regard to superior clinical efficacy of individual antipsychotics. In terms of adverse effect profiles, though, some antipsychotics are clearly more metabolically potent than others. Quite a few authors have pointed to a correlation of clinical improvement with the degree of metabolic adverse effects. Despite several important confounders, this apparent link is highly interesting in light of the essential role of lipid synthesis in the brain. Thus, understanding the mechanisms underlying adverse effects is not simply a step towards elimination of side effects, but may be necessary in order to develop more efficient antipsychotics. Judging by the lack of progress in such development during the last 60 years, the most realistic short-term aim will be to develop well-founded strategies for the prevention of weight gain, diabetes and dyslipidemia in patients treated with the drugs in question. As a complement to dietary measures and physical activity, metformin and statins appear to be the best prophylactic candidates.

10. Future perspectives

As mentioned in the Discussion, we recently published an article detailing the hypothalamic mechanisms underlying olanzapine-induced hyperphagia. An important question is whether the antipsychotics' lipogenic effects and negative effects on glucose metabolism are mediated via the CNS, or whether they occur due to direct effects of antipsychotics on peripheral metabolic tissues, such as the liver or adipose tissues. Distinguishing primary effects from feedback effects is challenging, and specific pharmacological and/or genetic inhibition seems a natural step forward in this regard. Such strategies may also strengthen the rationale for prophylactic pharmacological intervention for antipsychotic-induced metabolic adverse effects.

Further work is also necessary in order to increase the scientific and clinical relevance of rodent models in this field. In particular, issues such as drug dosing and administration should be carefully considered in order to reduce the gap between effects of olanzapine and clozapine, as well as gender differences, in rat. An improved rat model would facilitate *in vivo* investigations of the potential significance of antipsychotic-induced lipogenesis in myelination.

Regarding the translational aspects of our work, we plan to search for biological markers associated with the use antipsychotic drugs and metabolic adverse effects, in patient materials including several hundred patients who will be thoroughly examined clinically and biochemically. In one study, patients will be offered follow-up appointments for at least a year after starting randomized treatment with either olanzapine, amisulpride, or aripiprazole. We will have the opportunity to examine DNA and RNA profiles from these patients and, among other outcomes, investigate alterations in RNA expression during treatment initiation. Searching for alterations in the transcripts described in this thesis, as well as new candidates for further research, will be highly exciting. Correlating gene expression with clinical parameters may

enable the identification of biomarkers for clinical response, or for increased risk of metabolic adverse effects.

11. References

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